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14. ABSTRACT

The carcinogenic effects of low dose radiation are not clear. Moreover, it is not known if exposure to low dose radiation increases cancer risk in offspring of irradiated parents. Radiation is known to damage DNA and cause mutations. Cancer arises in part through mutations in oncogenes and tumor suppressor genes. However, epigenetics is also known to play an important and perhaps central role in cancer induction. Epigenetics is here defined as a heritable change in phenotype without an underlying mutational event. Epigenetic alterations may be induced early in development as a result of diet or environmental exposure. These alterations can persist into adulthood, and may increase the risk of developing diseases such as cancer later in life. Radiation has been shown to cause epigenetic effects in mammalian cells and in mice, although the role of these in human cancer is entirely unknown. Here we hypothesize that the carcinogenic effect of radiation may in part be due to epigenetic alterations. In this study we will determine if early exposure to radiation leads to a change in DNA methylation in adult tissues and in ensuing unexposed generations, and if these changes correlate with increased cancer susceptibility. We also expect to identify a "radiation exposure signature" which when validated, could be used clinically as a biomarker of radiation exposure. These experiments will also open up new avenues of investigation into the role of epigenetics in cancer caused by radiation and other environmental carcinogens.

15. SUBJECT TERMS
Epigenetic biomarkers, transgenerational, lung cancer

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Introduction

This study is designed to determine whether ionizing radiation results in changes to DNA methylation and if these changes are heritable, epigenetic changes. We hypothesize that exposure to low dose irradiation in utero will result in alterations to the pattern of DNA methylation and that some of these changes will then be passed on to future generations. We also hypothesize that these changes in methylation alter gene expression and may lead to increased rates of cancer. We are using a mouse model of in utero exposure and are focusing specifically on lung cancer.

Body

Pregnant mice at day 15 of gestation are exposed to a low dose (0.5Gy) of irradiation. The pups are either taken at day 10 (Aim 1), allowed age to 2 years (Aim 2) or used as breeders for subsequent generations (Aim 3). As the focus is irradiation and lung cancer we used the Balb/c strain of mouse which we have shonw in preliminary experiments to be susceptible to lung cancer after multiple low doses of irradiation.

We are collecting lung tissue, as well as liver, kidney, spleen, testes, ovary , heart, and plasma for possible future studies. We take a piece of each tissue for fixation in formalin and the rest of the tissue is snap frozen. Samples (except for plasma) for freezing are put into beem capsules and frozen in liquid nitrogen. Blood for plasma is collected and put into EDTA tubes, allowed to sit then centrifuged to remove blood cells. The supernatant (plasma) is then transferred into a cryotube and snap frozen. All frozen tissues are currently stored in a -80 freezer.

Although the focus of this study is on the male paternal line we are also collecting tissues from the female littermates and are collecting tissues from the maternal line. We will use these samples both for comparison to the males and for possible future studies.

Specific Aims

Aim 1- Changes in the DNA methylation between irradiated and non-irradiated 10 day old mice. All the samples for Aim 1 have been collected.

In our Sept 2011 report, we noted a potential difference in the body weight between the irradiated and control group of the male mice. To pursue this further, we collected mice from both the paternal lineage (F1,F2) and are now collecting from the maternal lineage. We found the weights of the males return to control levels by the F2 generation (Fig 1A). The body weights of the females also drop in the F0 irradiated group and also recover by the F2 generation in the paternal line. In the maternal line however the weights of the females continue to be reduced in the F2 generation (Fig 1B). We are still collecting mice from the maternal line for additional data points. The differences in effects in the F2 generation gives further support for examining the maternal line as well as the paternal.

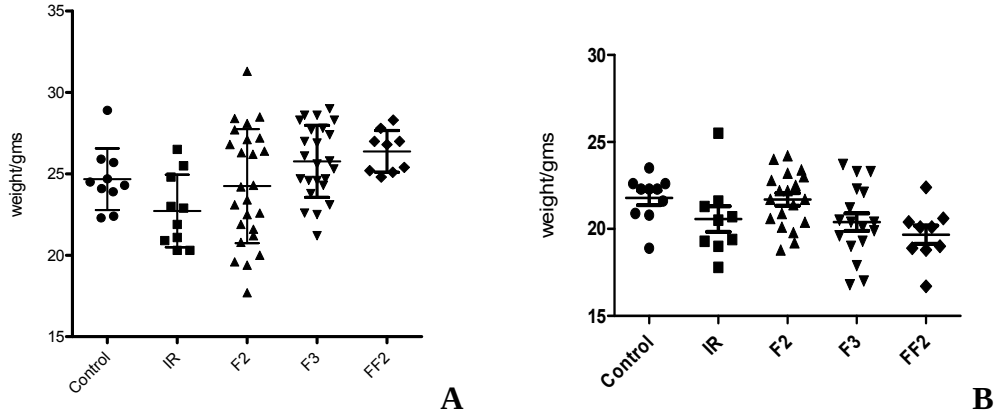

Figure 1 Body weight of 10 week old mice A.Males B. Females (Control, unirradiated; IR irradiated; FF2 is maternal transmission, all others are paternal transmission)

We have DNA methylation analysis on lung DNA from 10 week old mice. We initially a focused array analysis of genes implicated as methylated in lung cancer. The SAbiosystem EpiTect Methyl II PCR Array, mouse Lung Cancer Complete Panel contains 94 genes specifically found to be methylated in lung cancer. This platform uses qPCR technology, with primers designed around CpG islands known to be hyper-methylated in lung cancer. It does not require bisulfite conversion, instead uses methylation-sensitive and/or a methylation dependent restriction enzyme. We isolated DNA from 6 mice exposed to irradiation in utero and 6 control mice using a Qiagen DNeasy Kit. Samples were run on a Nanodrop for DNA yield and sent to Qiagen for analysis. In both groups of mice we observed hypermethylation of 7 genes: Cadherin 13 (Cdh13), HomeoboxA5 (Hoxa5), Cyclin dependent kinase inhibitor 1C (Cdknlc), Onecut domain FM2 (Onecut2), Stratifin (Sfn), Gata binding protein 6 (Gata6), and Tumor necrosis factor receptor superfamily member 25 (Tnfrsf25). DNA methylation of these seven genes even in the unirradiated group may explain the predisposition of these mice to lung tumors. Gata6, Tnfrsf25 and Sfn were all highly methylated >50% in both groups. There was a slight increase in DNA methylation of Tnfrsf25 and Sfn in the irradiated mice. Further study will include examination of these genes to determine if observed changes in DNA methylation translate to changes in gene expression.

Aim 2-Tumor development in the irradiated versus non-irradiated mice. We have generated both the irradiated and non-irradiated control mice for the tumor study (Table 1).

Mice for Aim 2	Total mice generated		# Mice Taken	
	M	F	M	F
Irradiated in utero	26	27	10	4
Control	33	36	9	1

Table 1. Numbers of mice for Aim 2

Mice are sacrificed when they show signs of distress, and are fully necropsied. Approximately half of the irradiated mice and a third of control have been taken to date (Table 1). The average survival rate thus far is 51 weeks for irradiated and 48.8 weeks for non-irradiated mice. A higher percentage of the irradiated male mice taken thus far had lung tumors at time of sacrifice than the irradiated controls. (Fig 2).

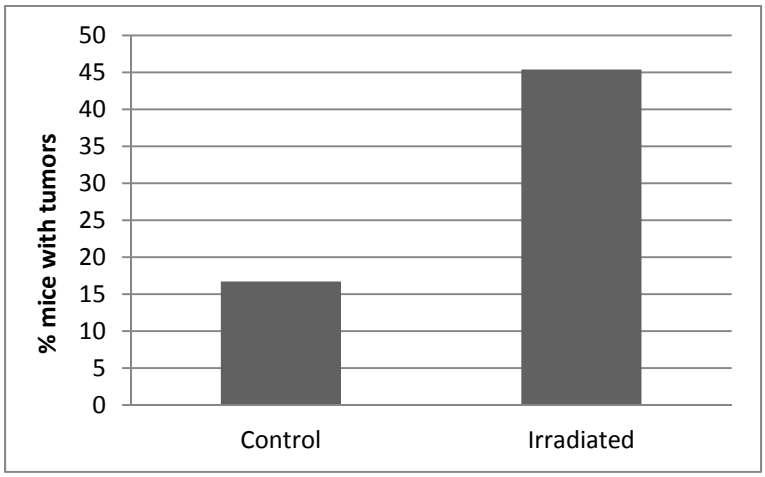

Figure 2. Percentage of irradiated vs control males with lung tumors at time of sacrifice

Fixed tissues from the irradiated group were stained by H&E to confirm the presence of lung tumors (Fig3).

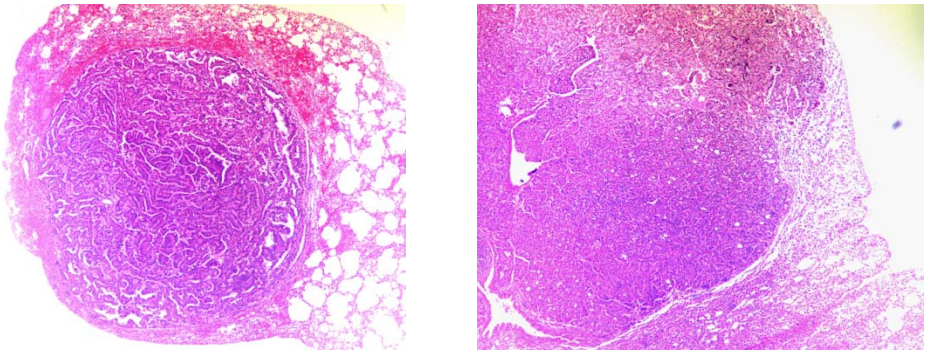

Figure 3. H&E staining of two lung tumors (40X)

We are continuing to monitor the mice and sacrificing them when they show signs of distress or at 2 years of age. Once all the mice have been sacrificed we will establish final survival curves, incidence of lung tumors and any other tumors. We will also use collected frozen lung tumor tissue for DNA methylation analysis

Aim 3-Transfer of epigenetic change through F2 and F3 generations. We have collected all the samples from mice in F2 and F3 generations from the paternal line. We have set up breeders and are collecting tissue from the maternal line as well (Table 2).

	Mice Generated		Mice Collected	
	M	F	M	F
Paternal				
F2	25	25	25	25
F3	21	16	21	16
Maternal				
F2	17	14	12	9
F3	6	9		

Table 2. Numbers of mice for Aim 3

We are continuing to generate mice for the maternal line. Once we have established an epigenetic profile from Aim 1 we will analyze the samples from the male F2 and F3 generations.

Key Research Accomplishments

- Collection of all samples for Aim 1
- Initial analyses of samples from Aim 1
- Generation of all mice needed for Aim 2 and preliminary analysis of tumor susceptibility.
- Start of collection and analysis of tissue from Aim 2
- Collection of all samples for paternal line for Aim 3
- Generation of mice and start of collection of maternal line for Aim 3

Reportable Outcomes

We have banked tissues from both male and female mice including liver, lung , kidney, and spleen for future studies.

In early 2012 our mouse room was put on quarantine due to pinworm contamination in another investigators mice. Although our mice never tested positive all mice were put on chow containing Fenbendazole from May 14 to Aug 1. During this time no live mice were allowed to leave the room. We do not anticipate any adverse outcomes from the diet but as no studies have been done on methylation changes due to Fenbendazole we cannot completely rule it out. Since both controls and irradiated groups were on the diet this should negate any effect on our results.

Conclusions

We have obtained evidence that in utero irradiation leads to reduced body weight in young adult mice and this may be transmitted to subsequent generations.

References

None.

Appendices

None.